THE STATES OF A GENE LOCUS IN MAIZE

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The locus of a gene whose action is governed by a known system of controlling elements may undergo a change that alters the pattern of expression of the gene in mature tissues. The change arises as a single event and thus resembles a mutation. Because the effect is on the gene-control mechanism, the alteration is not termed a mutation but rather a change in "state" of the locus. The states reflect the presence of a mechanism that is able to modify gene expression during development. The nature of the states and their significance will be discussed in this report.

The distinctiveness of the various states of a gene locus may be illustrated by selected examples. For this purpose, some of the states of $a_1^{m\text{-}1}$ and $a_1^{m\text{-}2}$ will be considered. These symbols were assigned to designate the effects on the A_1 (Anthocyanin) locus in maize of two independent inceptions of control of the action of its gene by the *Spm* (Suppressor-mutator) system. This action is required for production of anthocyanin pigment in both plant and kernel. The states of $a_1^{m\text{-}1}$ differ from those of $a_1^{m\text{-}2}$ in their modes of regulating gene action. Some aspects of the difference were mentioned in previous *Year Books,* but its breadth was not emphasized because knowledge of some of the states of $a_1^{m\text{-}2}$ was insufficient for characterization. Recent investigations have made it possible to compare a number of states of $a_1^{m\text{-}2}$ with those of $a_1^{m\text{-}1}$ previously studied. The comparisons demonstrate the manner in which a single control system provides diversity of regulation of gene expression. Although distinctions among states are made apparent in the phenotypes of both plant and kernel, only those manifested in the kernel will be considered here, as they can be readily illustrated.

The States of $a_1^{m\text{-}1}$

The states of $a_1^{m\text{-}1}$ were derived through modifications of the original state that arose when the first instance of inception of control of A_1 gene action by the *Spm* system occurred in my stocks. Each state was recognized initially by the appearance of an altered type or distribution of anthocyanin pigment in the progeny of a plant carrying the original state, and most often in a single individual of the progeny. Investigation of the cause of the changed phenotypes was begun with such individuals and continued with their progeny. The studies indicated that each change was initiated at the A_1 locus by the element of the control system residing there, and that it represented one type of this element's response to the *Spm* element, located elsewhere in the chromosome complement. In the presence of a fully active *Spm* element, the states of $a_1^{m\text{-}1}$ are distinguished from one another by differences in the time of "turning on" of A_1 gene action during development of a tissue, by the frequency of occurrence of such action in the cells of a tissue, and by the pigment intensity in those cells in which it is produced. They are also distinguished by the intensity of pigment produced in the

absence of an active *Spm* element.

One state produces no pigment when *Spm* is inactive. Each of the other states does produce pigment, which is uniformly distributed over the aleurone layer of the kernel. The pigment is intense with one state but pale to faint with the others. There is no direct relation between the expression given in the presence of a fully active *Spm* element and that given in its absence. It should also be mentioned that when two states are combined as alleles in a plant or kernel, gene action is regulated independently by each, and when an active *Spm* element is present, the pattern of anthocyanin distribution and intensity produced by one allele overlaps that produced by the other. Illustrations of the phenotypes of kernels produced by the different states of a_1^{m-1}, both in the presence and in the absence of a fully active *Spm* element, and the overlapping patterns produced by combinations of states as alleles, appear in the *Brookhaven Symposia in Biology*, Number 18.

Analysis of the states of a_1^{m-1} was not complicated. Simple rules could be formulated that allowed prediction of the phenotype each state would produce in response to changes in activity of the components of *Spm*. These components—component-1, the suppressor, and component-2, the mutator—were considered in *Year Book 64*. The distinctions among states relate to the gene-associated element of the system, which resides at the A_1 locus. How this element operates at the level of the gene and within the nucleus to accomplish such diverse modes of regulation of gene action is not yet known. It is important to recognize, nevertheless, that the gene-associated element of a two-element control system does provide a means for directing a broad range of types and patterns of gene expression during development.

The States of a_1^{m-2}

Some of the states of a_1^{m-2} are so multifaceted in expression that an understanding of them requires forms of analysis not demanded by any of the states of a_1^{m-1}. The information obtained from the analyses has provided additional evidence of the way in which gene action may be regulated differentially by a single system of controlling elements. The states of a_1^{m-2} may be divided into two major classes: those having an *Spm* element at or close to the A_1 gene locus, and those that show no evidence of the presence of *Spm* at the locus but respond to that element in a distinctive manner when it is located elsewhere in the chromosome complement. The original state, from which most of the other states were derived, belongs in the first class. An *Spm* element is present, either within or just distal to the locus of the structural gene(s).

The original state of a_1^{m-2}. The phenotype produced by the original state of a_1^{m-2} depends altogether on the phase of activity of each of the components of *Spm*. If component-1 is inactive, no pigment is produced in the plant or in the aleurone layer of the kernel. When this component is active, the gene is activated and pigment is produced. The type and distribution of the pigment depend on the activity of component-2. If this component is inactive, the aleurone layer is lightly pigmented. If component-2 acts only late in the development of a kernel, then small, deeply pigmented spots appear in a lightly pigmented background. If it is active at all stages of development, both large and small deeply pigmented spots appear in the lighter background. The kernels shown in Plate 1(A) illustrate responses of the initial state of a_1^{m-2} to these different phases of activity of the components of *Spm*.

The phenotype produced by this state does not depend solely on the action of the *Spm* element that is situated at or close to the locus. If either component of that element is inactive, a fully active *Spm* element located elsewhere in the chromosome complement will induce the same types of response.

The responses of the original state of a_1^{m-2} to phases of activity of component-1 of *Spm*, the suppressor or inhibitor component, are the reverse of those given by most of the states of a_1^{m-1}. Responses to component-2, the mutator component, are alike. The term "mutator" is applied to this component because it induces responses that modify the organization of the locus and thus the expression of the gene. One such modification is responsible for the origin of the different states that are under discussion in this report. Some states are produced that respond to component-1 of *Spm* but not to component-2. Others show no evidence of a response to component-1 but do respond to component-2. Still others, which respond to neither component, are termed "stable states," and each of them gives rise to a distinctive phenotype in both plant and kernel.

Only a few of the stable states derived from the original state of a_1^{m-2} resemble that of the A_1 locus before control of its gene action was taken over by the *Spm* system. They produce deep anthocyanin pigmentation in both plant and kernel, and in the kernel the pigment is uniformly distributed over the aleurone layer. Most of the stable states give rise to a distinctive pattern of pigment distribution and intensity in plant and kernel. In the kernels, clusters of cells with more intense pigment than the surrounding cells are distributed over the aleurone layer. The intensity of the background pigmentation ranges from faint with some states to deep with others. Three kernels with intermediate but differing levels of background pigmentation are shown in Plate 1(B), (C), and (D). Each of the stable states is inherited in the same manner as any stable mutant allele of the gene. Intralocus crossover studies have shown that the phenotype each produces relates to a component residing at a particular site within the locus.

New states, either responding or not responding to *Spm*, arise only from those states that react to component-2 of *Spm*. The event responsible for an altered state must occur in a cell of the germ line and the modified state must be included in a gamete. A zygote produced by the functioning of this gamete will give rise to a plant having the new state. The behavior of the state may then be analyzed in this plant and its progeny. With some states of both a_1^{m-1} and a_1^{m-2}, responses to component-2 of *Spm* occur only very late in development of a tissue. None may occur in the germ-line cells. Thus these states remain unaltered through successive plant generations even in the presence of an *Spm* element with an early-acting component-2. All states are inherited unaltered if component-2 is inactive or if its action is effective only very late in development.

The derived states of a_1^{m-2}. The phenotypes produced by four modified states of a_1^{m-2} appear in Plate 1(E). A fully active *Spm* element, placed at or close to the A_1 gene locus, is present in each kernel. No visible evidence of a response to component-1 of *Spm* is shown by any of these states. The dark background pigmentation in kernels like the one on the left, and the colorless backgrounds of kernels carrying the other three states, are the same regardless of the phase of activity of component-1 in the kernel. In the kernel on

the left the small deeply pigmented spots represent one response of the state present in the kernel to component-2. The size of such spots is always small. Another response to component-2 occurs early enough for the modified locus to be included in a number of gametes produced by a plant carrying this state. The modifications give rise to stable states that no longer respond to component-2. In the kernel, the aleurone layer is uniformly pigmented, and the intensity of color is the same as the background pigmentation produced by the parent state (the dark kernel in the photograph).

The second kernel from the left illustrates one of the responses of the state present in that kernel to component-2. The response produces pigmented areas, within which the pigment distribution is similar to that in the kernels shown in (B), (C), and (D). A second type of response is not visibly registered in the kernel but is made apparent by the stable derivatives this state produces. Some of them give rise to phenotypes resembling those in (B), (C), and (D). Others, however, produce no pigment in the aleurone layer of the kernel.

The state of $a_1^{m\text{-}2}$ present in the second kernel from the right is distinguished by the range of phenotypic expressions brought about by its responses to component-2. Areas in the aleurone layer exhibit phenotypes similar to those produced by several other states. One such area is visible in this kernel; it has many deeply pigmented spots in a pale-pigmented background. Other kernels carrying this state may have sharply defined areas containing a number of deeply pigmented spots in a colorless rather than a pale background. All the spots may be small, or some may be large and some small.

The kernel on the right in Plate 1(E) has only very pale spots in a colorless background. Some of the stable derivatives of this state give rise to kernels whose aleurone layer is uniformly pale-pigmented, but most of them produce colorless kernels.

Five additional states, each derived from the initial state, have been examined. None of them gives evidence of the presence of an *Spm* element at the locus of the A_1 gene. Each responds in a distinctive manner, however, to an active *Spm* element located elsewhere. The phenotypes produced by two of these states in the presence of a fully active *Spm* are shown in Plate 1(F). The two left-hand kernels have one state. Their deeply pigmented spots are distinguished from those in most of the other kernels illustrated by a near absence of diffusion rims. Usually, when deeply pigmented spots or areas are formed, a product that diffuses from the pigment-producing cells into the surrounding cells, and often through several rows of cells, allows pigment to be formed there even though the A_1 gene is not functioning in these cells.

The common expression given by the other state shown in Plate 1(F) is seen in the second kernel from the right. Small deeply pigmented spots appear in a colorless background. This state gives rise frequently to a new state, characterized by a marked increase in the number of pigmented spots, as in the adjacent kernel on the right.

The remaining three states have been examined in greater detail than most of the others, as they provide information about the operation of the *Spm* system that could not be deduced readily from the behavior of other states. Two of them, although independently isolated, are so much alike that they may be considered jointly. These states (7977B and 7995) furnished the initial evidence of the "presetting" and "erasure"

mechanism that was outlined and illustrated in *Year Book 63* (pp. 592–602) and further commented on in *Year Book 64* (pp. 527–536). This aspect of their behavior will not be restated here. Another aspect, not discussed earlier, will be considered in conjunction with the behavior of the remaining state, 8004. These three states differ greatly from the original state of $a_1^{m\text{-}2}$ in their responses to the changes in activity of the *Spm* element that can occur in individual cells during development of the endosperm of the kernel.

It is recognized that the components of *Spm* undergo changes in phase of activity. Control of the time and frequency of their occurrence resides in the *Spm* element itself, each change regulating in a distinctive manner the period when the next will take place. The changes in phase of activity of component-1, from active to inactive and back to active, are unambiguously registered by each of the states of $a_1^{m\text{-}1}$. The same unambiguity applies to the states of some of the other gene loci that have come under the control of the *Spm* system, and it applies to the original state of $a_1^{m\text{-}2}$. The phenotype that will appear after each phase change can be predicted and the predictions validated by tests that accurately determine the phase. Illustrations are given in the Brookhaven Symposium paper mentioned earlier. The states of $a_1^{m\text{-}2}$ designated 7997B, 7995, and 8004, however, respond in a distinctly different manner to the alternating phases of activity of *Spm*. The difference is especially well registered when the changes in phase occur during development of the kernel. State 8004 will be discussed first.

State 8004 of $a_1^{m\text{-}2}$. Examination of this state was conducted with plants having different constitutions with respect to the *Spm* element. These were: (1) no active *Spm* element; (2) one *Spm* element with both components fully active; (3) one *Spm* element with an active component-1 and a late-acting component-2; (4) two *Spm* elements, both as in (2); (5) two *Spm* elements, one as in (2) and one as in (3). In addition, some plants had an *Spm* element whose component-1 was inactive and remained inactive in most plants and in their progeny, returning to the active phase only very rarely; the returns could be observed in small regions within individual kernels. Numerous tests were conducted with plants having these different constitutions. For the purposes of this discussion, the evidence obtained from only a few kinds of test need be mentioned.

When state 8004 is propagated in the absence of an active *Spm* element, the aleurone layer of the kernel is colorless. If, however, an active *Spm* element is present, pigment is produced. Its type and distribution then depend on the nature of the *Spm* and the number of elements present. If component-2 of the *Spm* element(s) is inactive or becomes active only late in development, the aleurone layer is uniformly light-pigmented. If this component is active initially, different phenotypic expressions of the gene appear. When two or more such elements are present, pigment in the aleurone layer may range from nearly colorless in some kernels to dark pale in others, with, in most kernels, one or several very small deeply pigmented spots. If, however, a kernel starts development with a single active *Spm* element that undergoes change in phase of activity in some cells, early in development, then pigment intensities in the aleurone layer of the mature kernel are strikingly modified. Examples are seen in the kernels of Plate 2(A)–(D). These kernels have both large and small

areas outlined by rims of deep pigment. The background pigmentation in all but one kernel is faint: such kernels were purposely selected for clear illustration of the deeply pigmented borders. The kernel with a darker background (B) is included to suggest the range in intensity of background pigmentation among these kernels.

The rimmed areas consist of descendants of individual cells in which a change in phase of *Spm* activity has occurred. The deep pigment outlining an area is produced by the outermost cells of the area. They receive a diffusible substance from the cells surrounding the area, in which *Spm* is fully active. This substance allows the border cells to make a pigment that is more intense than that in the cells either inside or outside the area. Thus the deep pigment of the rims is the result of a complementation reaction. The rims indicate that the product of action of the A_1 gene (or genes) differs in the cells within and without the areas. The larger rimmed areas often contain small areas that also are rimmed with deep pigment. Again, the rims are the product of a complementation reaction. Here, however, the diffusible substance comes from cells within the small area and enters the cells surrounding it, where the deep pigment is produced. It could be demonstrated that the small rimmed areas within the larger ones are composed of descendants of cells in which *Spm* has returned to an active phase. Not all returns are made visible in this way. Some are accompanied by a change at the $a_1^{m\text{-}2}$ locus that alters its capacity to produce a substance that can complement.

In some kernels the pigment within a rimmed area is much more intense than that outside the area. It is not uniform in intensity, but mottled, as illustrated in Plate 2(C), (D). Some of the mottling is due to very small areas in which a diffusible complementing substance is produced. If a number of rimmed areas are present in a kernel, the intensity of the background pigmentation within the areas is often the same. In some kernels, however, it may differ in one or more of the areas; see Plate 2(B) and legend. It is suspected that the differences among kernels in the intensity of pigment within rimmed areas, exclusive of the rims, reflect initial differences in organization of the $a_1^{m\text{-}2}$ locus in the kernels, or differences that may arise during kernel development.

That such differences do arise was learned in tests conducted to determine the cause of interruptions of a rim along a segment of an otherwise rimmed area. Such interruptions, expressed as absence of deep pigment, are often noted. Some of them are not continuous but are arranged in sequence along a continuous segment. It was found that the interruptions are due in some instances to loss of ability of the adjacent outer cells to produce a complementing diffusible substance that will allow the border cells of the rimmed area to form deep pigment. The tests were made by introducing $wx^{m\text{-}8}$ along with state 8004 of $a_1^{m\text{-}2}$ into the primary endosperm nucleus.

The gene at the *Wx* (Waxy) locus functions to convert amylopectin into amylose in the starch granules of cells in the endosperm. Changes in action of this gene during development may be detected in the mature kernel visually and also quite precisely by staining the starch with an iodine–potassium iodide solution. Gene action at the $wx^{m\text{-}8}$ locus is under the control of the *Spm* system, and its responses to *Spm* result in the production of amylose in the

starch granules of the cells. Because all the cells of the endosperm below the aleurone layer—the outermost layer—have starch granules, each change in action of the gene during endosperm development is registered in the mature endosperm. The descendants of a cell in which a change has occurred form a well-defined sector in which the altered action of the gene is expressed in every cell. Most of the large sectors terminate in the aleurone layer. Small sectors, produced by changes occurring late in kernel development, also may terminate in the aleurone layer. Thus, when state 8004 of $a_1 m^{-2}$ and wx^{m-8} are both present in a kernel, the cell lineage of the aleurone layer overlying such a sector, either large or small, is also sharply defined. Parts of some of these sectors are adjacent to parts of a rimmed area, and sometimes these adjacent rim-area cells do not form deep pigment. The interruption of the rim is precisely defined by the common region of contact of cells of the sector with those of the rimmed area. It is evident that the sector is formed from descendants of a cell in which changes have occurred coincidentally at the loci of a_1^{m-2} and wx^{m-8}. The change at a_1^{m-2} alters its ability to make a diffusible substance that can be utilized by the adjacent cells in the rimmed area to form intense pigment.

In *Year Book 57* the first evidence of phenotypic change produced by alternating cycles of activity of *Spm* was outlined. The studies were conducted with a selected state of a_2^{m-1}. (A_2 is another locus in maize whose gene is involved in the biosynthetic pathway leading to anthocyanin formation.) It was learned that the number and size of pigmented areas in a kernel, produced as the consequence of a change of *Spm* from an active to an inactive phase, depend on the number of *Spm* elements present in the kernel. When one is present, many areas exhibit this change, and many of them are large. With two *Spm* elements, the areas are all small. With three elements, no areas or only some very small ones are formed. The same relationship governs the production of the rimmed areas with state 8004 of a_1^{m-2}. Few or no rimmed areas are formed if two or more active *Spm* elements are present in the kernel. Both large and small areas are produced if only one *Spm* element is present.

An additional aspect of the behavior of state 8004 should be mentioned. If a plant carrying this state and also one active *Spm* element is utilized as pollen parent in a cross to a plant that lacks an active *Spm* element, the kernels receiving the *Spm* element from the pollen parent may show large rimmed areas, and in some kernels the pigment within the areas is dark. Kernels that do not receive the *Spm* element from the pollen parent are colorless. Removal of *Spm* from the nucleus by meiotic segregation does not induce a setting of the locus that allows pigment of various intensities to be produced subsequently among the kernels, as happens with states 7997B and 7995.

States 7997B and 7995 of a_1^{m-2}. An earlier report (*Year Book 63,* pp. 592–602) showed that kernels having one of these states and also one or more fully active *Spm* elements develop a number of small, deeply pigmented spots in a more lightly pigmented background. Should only one *Spm* element be present and should this element undergo change in phase of activity during kernel development, the response of either state to the change results in a darkly pigmented area in the aleurone layer. Many of these dark areas contain small colorless areas. Examples are

seen in the two kernels in Plate 2(E), each of which carries state 7995. Kernels with a faintly pigmented background were chosen for the illustration, so that the dark areas might be noted readily. In the parts of the kernels where the *Spm* element was active, the characteristic pattern of small, deeply pigmented spots appears. Such spots are absent in the darkly pigmented areas. Except for the small colorless spots, the pigment within the dark areas is uniform in intensity. The edges of the areas are not defined by deeply pigmented rims, as are comparable areas in kernels carrying state 8004. Rims will appear if the two states 8004 and 7995 are present as alleles in a kernel. An example is shown in Plate 2(F). Here the uniformly dark areas, produced by state 7995, are bordered by deeply pigmented rims, produced by state 8004. The small colorless spot within the area on the left is also bordered by a pigmented rim resulting from the action of state 8004.

The pigment produced by state 7995 when a change in phase of *Spm* activity occurs during kernel development differs markedly both in intensity and in pattern of distribution from that produced by this state when *Spm* is removed by a somatically occurring transposition of *Spm* or by means of meiotic segregation. In a kernel that develops from the functioning of a gamete that has lost the *Spm* element by such means, the aleurone layer has a distinctively different pattern of pigment distribution, as illustrated in *Year Book 63* (pp. 592–602 and Plate 2).

This review of the states of a gene locus is intended to illustrate the extraordinary diversity in capacity of a single system of controlling elements to regulate the action of a gene during development. It also demonstrates that the distribution and degree of expression of the end product of action of a series of genes is mediated through control of the action of one of these genes. Only the fact that anthocyanin pigment—the end product of such a series—is not vital to the plant makes it possible to learn about the many kinds of regulation such a system can provide. It is well known that the various races and strains of maize are distinguished from one another by a remarkable diversity with respect to distribution of anthocyanin to parts of the plant and its intensity in any one part. The patterns are so varied that they defy a meaningful classification. The same is true of the different distributions of pigment produced by the states of a_1^{m-1} and, especially, the states of a_1^{m-2}. Studies conducted by maize geneticists have shown that in some instances different alleles of a gene locus involved in anthocyanin production are responsible for the appearance of different patterns of pigment distribution. The kind of regulation exercised by some of these alleles resembles that afforded by some of the states described here. In the studies of the alleles, with two exceptions, it has not been possible to determine the presence at the gene locus of a control-mechanism component that could be responsible for the differences in action of the alleles. That is understandable, for a means of detecting such a component usually is not available. It can be suspected, however, that many of these alleles represent different "states" of the loci, in the sense of the term defined in this report. Without a means of distinguishing between a mutant of the structural gene itself and a mutant that is produced by a regulatory component at the locus, the term "allele" must be retained even though its significance in any one instance will remain ambiguous.

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